

In the Claims

Claims 1-3 (Canceled)

4. (Currently amended) The method of claim 23 wherein said support matrix is a ~~gel, sol-gel,~~ scaffold, ~~hydrogel,~~ sponge, honeycomb or lattice prepared from a compound selected from the group consisting of a ~~thermo-reversible gelation hydrogel, collagenous gel,~~ Type I collagen, Type II collagen, Type IV collagen, gelatin, agarose, collagen containing proteoglycan, collagen containing glycosaminoglycan, collagen containing glycoprotein, collagen containing fibronectin, collagen containing laminin, collagen containing a growth factor, collagen containing cytokine, collagen containing elastin, collagen containing hyaluronin, collagen containing fibrin, collagen containing a synthetic polymeric fiber made of polylactic acid, collagen containing a synthetic polymeric fiber made of polyglycolic acid, collagen containing a synthetic polymeric fiber made of polyamino acid, collagen containing a polycaprolactone, collagen containing a polyamino acid, collagen containing a polypeptide-gel, a copolymer thereof and a combination thereof.

5. (Currently amended) The method of claim ~~[[4]]~~ 23 wherein said support matrix is ~~the~~ a thermo-reversible gelation hydrogel (TRGH) wherein said TRGH is in a liquid sol state at temperatures of below about 30°C and wherein said thermoreversible hydrogel polymer is in a solid sol state at temperature above about 30°C and further wherein said thermo-reversible gelation hydrogel is either deposited into ~~the~~ a lesion cavity formed below the top sealant or between the bottom and top sealants as the ~~neo-cartilage~~ construct comprising chondrocytes ~~embedded~~ seeded therein or wherein

said TRGH is deposited into said cavity as a space holding gel without any ~~neo-cartilage~~ chondrocytes.

6. (Currently amended) The method of claim ~~[[5]]~~ 23, wherein said ~~autologous or heterologous~~ chondrocytes of step b) are expanded ~~cultured~~ and differentiated in a cell culture.

7. (Currently amended) The method of claim 24 wherein said bottom sealant is selected from the group consisting of gelatin, a copolymer of polyethylene glycol and poly-lactide or poly-glycolide, periodate-oxidized gelatin, 4-armed pentaerythritol thiol and a polyethylene glycol diacrylate, 4-armed tetra-succinimidyl ester or tetra-thiol derivatized PEG, photo-polymerizable polyethylene glycol-co-poly(α -hydroxy acid) diacrylate macromer, 4-armed polyethylene glycol derivatized with succinimidyl ester and thiol further cross-linked with methylated collagen, derivatized polyethylene glycol (PEG), polyethylene glycol (PEG) cross-linked with alkylated collagen, tetra-hydrosuccinimidyl or tetra-thiol derivatized PEG, PEG cross-linked ~~PEG~~ with methylated collagen, and a combination thereof.

8. (Currently amended) The method of claim 7 wherein the bottom sealant is the PEG cross-linked ~~PEG~~ with methylated collagen.

9. (Currently amended) The method of claim 8 wherein the ~~neo-cartilage~~ construct is prepared *in vitro*, *ex vivo* or *in vivo*.

Claims 10-12 (Canceled)

13. (Currently amended) The method of claim ~~[[12]]~~ 23 wherein the hydrostatic cyclic pressure is from about 0.05 MPa to about 3 MPa at 0.1 to about 0.5 Hz or the hydrostatic constant pressure is from about ~~zero~~ 0.05 MPa to about 3 MPa above atmospheric pressure and wherein such pressure is applied for about 7 to about 28 days.

14. (Currently amended) The method of claim 13 wherein said hydrostatic pressure is ~~preceded or~~ followed by a period of about ~~zero~~ one to about 28 days ~~of~~ at an atmospheric pressure.

15. (Original) The method of claim 14 wherein said perfusion flow rate is from about 5 μ L to about 50 μ L/minute.

16. (Original) The method of claim 15 wherein said perfusion flow rate is about 5 μ L/minute.

17. (Original) The method of claim 16 wherein said perfusion and pressure are applied at from about 2% to about 5% of oxygen concentration.

Claims 18-22 (Canceled)

23. (Currently amended) A method for treatment of a cartilage lesion and for formation of a superficial cartilage layer, comprising steps:

a) obtaining an autologous or heterologous cartilage and subjecting said cartilage to a process for isolating of chondrocytes or providing cells that could be differentiated into chondrocytes;

b) expanding and suspending said isolated chondrocytes in a collagen, collagen gel, collagen sol, ~~sol-gel, collagen~~ or a collagen-containing solution;

c) seeding said chondrocytes suspension into a support matrix, wherein said support matrix is a three-dimensional structure containing plurality of pores, thereby producing a seeded matrix ~~construct~~;

d) preparing a ~~neo-cartilage~~ construct for implantation into said cartilage lesion by subjecting said seeded support matrix ~~construct~~ to conditions promoting activation and propagation of said chondrocytes within said support matrix wherein said conditions for activation and propagation of chondrocytes comprise a cyclic or constant hydrostatic pressure, a static atmospheric pressure, flow rate of a culture medium, temperature under which said activation and propagation is performed, length of time, cell density, oxygen or carbon dioxide content, each alone or in combination,

wherein the hydrostatic pressure is from about ~~zero~~ 0.001 MPa to about 10 MPa above atmospheric pressure at about 0.01 to about 1 Hz, wherein the time for applying the hydrostatic pressure is from ~~zero~~ about 1 to about 8 24 hours per day for from about one day to about ninety days, wherein said hydrostatic pressure is ~~preceded or~~ followed by a period of ~~zero~~ 16 to about 24 23 hours per day of a static atmospheric pressure for from about one day to about ninety days, wherein the flow rate is from about 1 $\mu\text{L}/\text{min}$ to about 500 $\mu\text{L}/\text{min}$, ~~wherein the cell density is from about 3 to 60 millions~~ and wherein the oxygen concentration is from about 1 to about 20%;

e) implanting said ~~neo-cartilage~~ construct into said cartilage lesion; and

f) depositing ~~the~~ a top ~~biocompatible~~ adhesive sealant over said ~~the neo-cartilage~~ construct wherein said top sealant is ~~the~~ polyethylene glycol cross-linked with methylated collagen,

wherein said deposition of said top sealant over said implanted ~~neo-cartilage~~ construct results in formation of the

superficial cartilage layer that overgrows and ~~protects~~ said ~~neo-cartilage~~ construct implanted within said lesion.

24. (Currently amended) The method of claim 23 additionally comprising a step of depositing a layer of a bottom ~~biocompatible~~ adhesive sealant into said cartilage lesion before implanting said ~~neo-cartilage~~ construct, wherein said sealant may be the same as, or different from the top sealant.

Claims 25-26. (Canceled)

27. (Currently amended) The method of claim ~~[[26]]~~ 24 wherein said superficial cartilage layer is integrated into a synovial membrane.

Claims 28-29. (Canceled)

30. (Currently amended) A method for treatment of a cartilage lesion and for formation of a superficial cartilage layer, said method comprising steps:

a) obtaining an autologous or heterologous cartilage and subjecting said cartilage to a process for isolating of chondrocytes or providing cells that could be differentiated into chondrocytes;

b) expanding and suspending said isolated chondrocytes in a collagen, collagen gel, collagen sol, ~~sol-gel~~, ~~collagen~~ or a collagen-containing solution;

c) seeding said chondrocytes suspension into a support matrix, wherein said support matrix is a three-dimensional structure containing plurality of pores, thereby producing a seeded matrix ~~construct~~;

d) preparing a ~~neo-cartilage~~ construct for implantation into said cartilage lesion by subjecting said seeded support

matrix ~~construct~~ to conditions promoting activation and propagation of said chondrocytes within said support matrix wherein said conditions for activation and propagation of chondrocytes comprise a cyclic or constant hydrostatic pressure, a static atmospheric pressure, flow rate of a culture medium, temperature under which said activation and propagation is performed, length of time, cell density, oxygen or carbon dioxide content, each alone or in combination,

wherein the hydrostatic pressure is from about ~~zero~~ 0.01 MPa to about 10 MPa above atmospheric pressure at about 0.01 to about 1 Hz, wherein the time for applying the hydrostatic pressure is from ~~zero~~ about 1 to about ~~24~~ 8 hours per day for from about one day to about ninety days, wherein said hydrostatic pressure is ~~preceded or~~ followed by a period of ~~zero~~ 16 to about 23 ~~24~~ hours per day of a static atmospheric pressure for from about one day to about ninety days, wherein the flow rate is from about 1 $\mu\text{L}/\text{min}$ to about 500 $\mu\text{L}/\text{min}$, ~~wherein the cell density is from about 3 to 60 millions~~ and wherein the oxygen concentration is from about 1 to about 20%;

e) depositing a layer of a bottom adhesive sealant into said cartilage lesion before implanting said ~~neo-cartilage~~ construct;

f) implanting said neo-cartilage construct into said cartilage lesion; and

g) depositing a layer of a top adhesive sealant over the ~~neo-cartilage~~ construct wherein said top sealant is the polyethylene glycol cross-linked with methylated collagen,

wherein said bottom and top sealant may be the same or different,

wherein said deposition of said top sealant over said implanted ~~neo-cartilage—construct~~ construct results in formation of the superficial cartilage layer that overgrows and protects said ~~neo-cartilage~~ construct implanted within said lesion.

31. (Previously presented) The method of claim 30 wherein said top or bottom sealant is selected from the group consisting of gelatin, a copolymer of polyethylene glycol and poly-lactide or poly-glycolide, periodate-oxidized gelatin, 4-armed pentaerythritol thiol and a polyethylene glycol diacrylate, 4-armed tetra-succinimidyl ester or tetra-thiol derivatized PEG, photo-polymerizable polyethylene glycol-co-poly(α -hydroxy acid) diacrylate macromer, 4-armed polyethylene glycol derivatized with succinimidyl ester and thiol, alone or further cross-linked with methylated collagen, derivatized polyethylene glycol (PEG), polyethylene glycol (PEG) cross-linked with alkylated collagen, tetra-hydrosuccinimidyl or tetra-thiol derivatized PEG, PEG cross-linked with methylated collagen and a combination thereof.

32. (Previously presented) The method of claim 31 wherein said top or bottom sealant is the polyethylene glycol cross-linked with methylated collagen or wherein both, the top and bottom sealant, are polyethylene glycol cross-linked with methylated collagen.

33. (Currently amended) The method of claim 32 wherein the hydrostatic cyclic pressure is from about 0.05 MPa to about 3 MPa at 0.1 to about 0.5 Hz or constant pressure is from about ~~zero~~ 0.05 to about 3 MPa above atmospheric pressure ~~and wherein such pressure is applied for about 7 to about 28 days.~~

34. (Canceled)

35. (Previously presented) The method of claim 34 wherein said perfusion flow rate is from about 5 μ L to about 50 μ L/minute.

36. (Previously presented) The method of claim 35 wherein said perfusion flow rate is about 5 μ L/minute.

37. (Previously presented) The method of claim 36 wherein said perfusion and pressure are applied at from about 2% to about 5% of oxygen concentration.